Phenotypic and molecular detection methods for carbapenemase-producing organisms and their clinical significance at two Scottish tertiary care hospitals

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KEYWORDS
Carbapenemase-producing organisms
Carbapenem-producing Enterobacteriaceae
Carbapenem resistance
Molecular diagnostics
Antimicrobial resistance

ABBREVIATION LIST
AMRHAI: Antimicrobial Resistance and Healthcare Associated Infections
BLA: beta-lactamase
CPO: carbapenemase-producing organism
EUCAST: European Committee on Antimicrobial Susceptibility Testing
IMP: imipenemase
MIC: minimum inhibitory concentration
NDM: New Delhi metallo-beta-lactamase
OXA: oxacillinase
PCR: polymerase chain reaction
VIM: Verona integron-encoded metallo-beta-lactamase
ABSTRACT

Purpose
This study evaluated in-house PCR testing for local identification of bacteria carrying the major carbapenemase genes (bla_{OXA-48-like}, bla_{VIM}, bla_{NDM}, bla_{KPC} and bla_{IMP}).

Methodology
Carbapenemase-producing organisms (CPO) isolated from patients managed in two tertiary care hospitals in Scotland from September 2014–January 2017 were investigated. A combination of chromogenic screening agar (ChromID CARBA SMART\textsuperscript{TM}), a carbapenem hydrolysis test (Rapidec Carba NP\textsuperscript{TM}) and in-house real-time PCR for the bla_{OXA-48-like}, bla_{VIM}, bla_{NDM}, bla_{KPC} and bla_{IMP} genes were utilised. All isolates were sent to the AMRHAI reference unit for confirmatory testing.

Results
During the 29-month study period 39 CPO were isolated from 34 patients. The average turnaround time for a workflow involving phenotypic and molecular testing was 4.2 days. PCR had a sensitivity and specificity of 100%. The most common carbapenemase genes were bla_{OXA-48-like} (31%), bla_{VIM} (23%) and bla_{NDM} (20%). Resistance to antimicrobials other than beta-lactams was common; the most active agents were colistin, amikacin and fosfomycin. 27 patients were considered colonised (though CPO detection influenced empiric antimicrobials in five) and a CPO was implicated in infection in seven patients (bacteraemia in immunocompromised patients, n=2; surgical site infections, n=2; osteomyelitis in a patient with diabetes mellitus; and urinary tract infections, n=2). All patients survived infection.

Conclusion
In a low incidence setting we demonstrate the efficacy of a combined local laboratory workflow for rapid detection of CPO, incorporating phenotypic and molecular testing. In 7/34 patients the CPO was implicated as a pathogen and detection influenced antimicrobial decision-making in 5 colonised patients.
INTRODUCTION

Over the last decade, carbapenemase-producing organisms (CPO) have emerged as a major antimicrobial resistance concern. The frequent co-existence of resistance to additional antimicrobial classes can make management of CPO infections extremely challenging. Emphasising the clinical significance of these organisms is the observed association between carbapenem minimum inhibitory concentration (MIC) and mortality in Gram negative bacteraemia (1). The prevalence of CPO is highest in Southern Europe and Asia, but international travel has facilitated their global dissemination, with detection reported worldwide and the prevalence likely under-estimated due to a lack of systematic surveillance in many countries (2, 3). Although CPO detection is low in Scotland relative to the rest of the U.K. (61 vs. 1893 isolates in 2015), surveillance data from the Public Health England Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) reference unit demonstrates a steady increase since 2003 (when there was a single isolate in Scotland), underscoring the need to develop diagnostic capabilities (4).

Routine CPO screening in our institution is performed on patients who have been hospitalised outwith Scotland in the last 12-months, had contact with a CPO-colonised patient, or been previously colonised. If a CPO is detected in a clinical area then the other patients are screened weekly until the positive patient has been discharged. In addition, if a patient is admitted from another unit with a recent CPO then screening will also be performed. Screening utilises a rectal swab and/or faecal specimen, catheter urine if catheterised and wound swab/sputum if relevant. In high risk patients three negative screens are required to demonstrate negativity and allow stepping down of patient isolation and enhanced contact precautions. Current practice in our microbiology laboratory utilises phenotypic detection methods to detect CPO, with subsequent reference laboratory confirmation (Figure 1a).

Molecular detection of carbapenemase genes performed locally, prior to testing by a regional/national reference laboratory, offers the advantage of rapid confirmation of CPO identity. The aims of this study were to (i) evaluate the use of in-house real-time multiplex PCR testing for identification of organisms carrying the major carbapenemase genes (bla\textit{OXA-48-like}, bla\textit{VIM}, bla\textit{NDM}, bla\textit{KPC} and bla\textit{IMP}) as part of the local laboratory workflow and (ii) describe the clinical significance of CPO isolates detected at our institutions as part of laboratory, infection control and clinical quality improvement, using routinely gathered data.

METHODS

CPO isolates

CPO isolated from patients managed in two tertiary care hospitals in Edinburgh, U.K., between September 2014–January 2017, were identified retrospectively. All isolates were also sent to AMRHAI for confirmation of carbapenemase status by MIC determination (agar dilution) and molecular testing. This was considered the ‘gold standard’ (5, 6). Organisms investigated for carbapenemase production (Figure 1a) were either identified from screening media (ChromID CARBA SMART\textsuperscript{TM}, Biomerieux) or from routine clinical specimens found by the Vitek2 automated susceptibility testing analyser (Biomerieux) to have a raised carbapenem MIC (meropenem ≥0.5mg/L and/or ertapenem ≥1mg/L; the lowest possible result for both generated by the Vitek2).

Laboratory workflow

Isolates arising from routine susceptibility testing of clinical specimens were examined further by performing a meropenem E-test (Biomerieux) on Mueller-Hinton agar (Oxoid) and applying the same 0.5 McFarland standard inoculum to both halves of a chromID CARBA SMART\textsuperscript{TM} bi-plate. After 18-24 hours incubation in an aerobic atmosphere at 37°C, any isolates either...
growing on the screening agar and/or with a meropenem E-test MIC >0.125 mg/L (per EUCAST guidelines(7)) were further investigated by Rapidec Carba NP™ (Biomerieux) and in-house PCR.

Isolates identified from growth on screening media were further examined by Rapidec Carba NP™ testing, a meropenem E-test and Vitek2 automated susceptibility testing. If the Vitek2 meropenem MIC was ≥0.5mg/L, the meropenem E-test MIC was >0.125 mg/L or the Rapidec Carba NP™ was positive, then in-house PCR was performed.

**Phenotypic testing methods**

The ChromID CARBA SMART™ bi-plate comprises one half specific for bla\textsubscript{OXA-like} producing organisms while the other half is specific for bla\textsubscript{VIM}, bla\textsubscript{NDM}, bla\textsubscript{KPC} and bla\textsubscript{IMP} producing organisms. The Rapidec Carba NP™ is a ready-to-use strip and employs the principles of the Carba NP™ test, a novel phenotypic carbapenem hydrolysis test (8) which relies on \textit{in vitro} hydrolysis of a carbapenem by a bacterial lysate which is then detected by an alteration in pH using a phenol red indicator. The process including organism lysis and test incubation takes 2.5 hours.

**Molecular testing methods**

Isolates were subjected to a simple boil extraction and then analysed by quadruplex real-time PCR for bla\textsubscript{OXA-48-like}, bla\textsubscript{VIM}, bla\textsubscript{NDM} and bla\textsubscript{KPC} genes and monoplex real-time PCR for bla\textsubscript{IMP} (primer/probe sequences in Supplementary Table 1). These in-house PCRs were performed on the CFX Touch (BioRad) and results were analysed and interpreted using the BioRad CFX Manager software. The extraction process took 15 minutes and the PCR thermocycling component 2 hours.

**RESULTS**

**Phenotypic and molecular detection of carbapenemase-producing organisms**

During the 29-month study period 81 isolates were investigated (Figure 1a) and 39 CPO were identified. The chromogenic CPO screening agar (ChromID CARBA SMART™) had a sensitivity of 97.4% and a specificity of 95.2% for detection of CPO (38/39 carbapenemase positive and 40/42 carbapenemase negative isolates were confirmed; the false negative result was a bla\textsubscript{OXA-48-like}-producing \textit{E. coli}). 38/39 carbapenemase-positive and 41/42 carbapenemase-negative isolates were confirmed using the Rapidec Carba NP™ test, giving a sensitivity of 97.4% and a specificity of 97.6% (the false negative result was a bla\textsubscript{OXA-48-like} producing \textit{E. coli}). Two bla\textsubscript{OXA-23}-producing \textit{Acinetobacter baumannii} and one bla\textsubscript{IMP}-producing \textit{Enterobacter cloacae} were positive by Rapidec Carba NP™ and were characterised by AMRHAI. The sensitivity and specificity of the in-house multiplex real-time PCR was 100% and 100% (36/36 carbapenemase-positive isolates and 42/42 carbapenemase-negative isolates confirmed). Details of carbapenemase genes identified and organisms carrying these are shown in Figures 1b and 1c. The average turnaround time from specimen receipt to confirmation of CPO type was 4.2 days using the described workflow and 12.2 days if sent to the reference unit.

**Patient demographics**

The 39 CPO isolates were obtained from 34 different patients. 20/34 patients were male and the median age was 62 years (interquartile range 49-71). Seventeen patients were taking acid suppressing medication at the time of CPO isolation. Three patients had been re-patriated from hospitals abroad (Nigeria, India and South East Asia). A further five patients had recently travelled abroad (Turkey, n=2; India & China, n=1; India, n=2).
All \( \text{bla}^{\text{IMP}} \)-carrying organisms were isolated from patients in the Haematology (n=4) or Oncology (n=1) wards, with no recent travel history. Four of these isolates underwent PFGE typing by the AMRHAI. Two were unique but two were identical and these came from two inpatients on the same ward during an overlapping time period, supporting patient-to-patient transmission (though not excluding a common environmental source).

Of all 39 CPO isolates, 19 were detected from CPO screening specimens (during the study period 9057 screening specimens were examined) and 20 were first detected from a clinical specimen, Table 1. Of the 20 organisms isolated from clinical specimens, four were detected on a concomitant screen (performed in 16 cases).

**Carriage over time**

Subsequent specimens (screening or repeat clinical) were obtained in 21/39 patients and in 17 cases did not identify a CPO. Of the 20 patients with a CPO initially identified from a clinical specimen, 12 had the specimen type repeated and in three cases (two, three and seven months later) the original CPO was still present. In the 19 patients with a CPO isolated from a screening specimen, nine had screening repeated afterwards, two of which were positive (1 and 20 months later).

**Clinical significance**

In 22 patients, the isolation of a CPO was interpreted as colonisation and did not influence antimicrobial therapy. In five patients, the isolates were interpreted as colonisation but influenced empiric treatment of infection until additional microbiology results were available (n=3), empiric therapy of an infection with no diagnostic microbiology (n=1), and led to eradication therapy (urine colonisation) in one immunosuppressed patient.

In seven patients the detection of a CPO was considered to represent infection and all of these organisms were identified from clinical specimens (details in Table 2).

**Antibiograms**

The complete antibiogram (derived from Vitek2 system or superseded by reference laboratory result in cases of disagreement) of CPO isolates from this study is presented in Figure 2. Across all isolates, the most active antimicrobials were colistin (95% susceptible), amikacin (74% susceptible), fosfomycin (74% susceptible) and tigecycline (63% susceptible). Ciprofloxacin and gentamicin did not have reliable activity (31% and 49% respectively).

**DISCUSSION**

Three carbapenemase detection methods were utilised in this study: chromogenic screening agar, a carbapenem hydrolysis test and in-house real-time PCR for the \( \text{bla}^{\text{OXA-48-like}} \), \( \text{bla}^{\text{VIM}} \), \( \text{bla}^{\text{NDM}} \), \( \text{bla}^{\text{KPC}} \) and \( \text{bla}^{\text{IMP}} \) genes. All methods had sensitivities of 97.4% to 100% and specificities of 95.2% to 100%, with PCR achieving 100% for both. The importance of including the carbapenem hydrolysis test is underscored by the detection of two \( \text{bla}^{\text{OXA-23}} \)-carrying isolates and a \( \text{bla}^{\text{IMI}} \)-carrying isolate as these targets were not included in the PCR panel, highlighting an inherent limitation to targeted PCR. The validated in-house PCR for the ‘Big 5’ carbapenemase genes is now included in our local laboratory portfolio for carbapenemase detection and we contend that there is an important role for locally performed PCR for detection/exclusion of CPO. This will enable early initiation of appropriate infection control measures and clinical decisions in the case of a positive result and for infection control measures to be de-escalated and side rooms released in the case of negative results. We
also recognise the emerging role for new immunochromatographic lateral flow assays as rapid
diagnostic tests for identification of carbapenemase enzymes (9).

For carbapenemase screening, EUCAST recommend using a meropenem MIC cut-off of
>0.125mg/L. In this study, further CPO investigations were performed on routine clinical
specimens if the Vitek2 meropenem MIC was ≥0.5mg/L, the lowest possible value for this
system. Since this MIC cut-off is higher than the EUCAST recommendation, the screening
step of our workflow may under-detected carbapenemase production by clinical isolates.

34 patients had a CPO identified from a screening or clinical specimen. Interestingly, a low
proportion of CPO isolated from clinical specimens were detected on a concomitant screening
specimen (4/16). Recognised risk factors of travel to/hospitalisation in high CPO prevalence
countries and proton pump inhibitor usage were common (10). PFGE typing indicates a
possible nosocomial transmission event may have occurred between two patients.

Seven patients had infection involving a CPO with no infection related deaths and combination
therapy used in 3/7 cases (Table 2). The clinical outcomes for patients in our small case series
differ from results of a pooled analysis of 692 patients with carbapenemase-producing
Enterobacteriaceae infection published in 2014 (11). This large cohort was heterogeneous in
terms of host immune status, organism and resistance gene (though K. pneumoniae was
commonest), presence of bacteraemia and requirement for intensive care unit admission.
Various mortality outcomes were reported (including in-hospital, 30-day, infection-related),
with values of up to 80% in some studies, with benefit of combination therapy over
monotherapy in critically ill patients with bacteraemia.

In summary, in a low incidence Scottish setting we demonstrate the efficacy of a combined
laboratory workflow for local detection of CPO, incorporating chromogenic screening agar, a
carbapenem hydrolysis test and PCR assay for the \( \text{bla}_{\text{OXA-48-like}}, \text{bla}_{\text{VIM}}, \text{bla}_{\text{NDM}}, \text{bla}_{\text{KPC}} \) and \( \text{bla}_{\text{IMP}} \)
genotypes. Screening and clinical specimens contributed almost equally to the 39 CPO isolates
identified over the 29-month study period. In seven cases the CPO was implicated in causing
infection.

**FUNDING INFORMATION**

This work received no specific grant from any funding agency.

**ACKNOWLEDGEMENTS**

We would like to thank colleagues from the PHE Antimicrobial Resistance and Healthcare
Associated Infections (AMRHAI) Reference Unit, Colindale, London, NHS Lothian Clinical
Microbiology laboratory, NHS Lothian Infection Prevention and Control Team, Dr. Ewan Olson
and Dr. Kristjan Helgason.

**CONFLICTS OF INTEREST**

We declare there are no conflicts of interest.

**REFERENCES**

   of clinical outcomes in patients with bloodstream infections due to Gram-negative bacteria
2. Nordmann P, Naas T, Poirel L. Global spread of Carbapenemase-producing
Figure 1: Laboratory methods and carbapenemase-producing organisms identified

a Flowchart illustrating laboratory workflow.

b Carbapenemase genes identified. $\text{bla}_{\text{IMI}}$ and $\text{bla}_{\text{OXA-23}}$ genes determined by the reference laboratory (AMRHAI, Colindale).

c Organisms carrying carbapenemase genes

- In-house PCR targets
  - Enterobacteriaceae:
    - E. cloacae
    - E. coli
    - K. pneumoniae
    - C. freundii
  - Non-enterobacteriaceae:
    - A. baumannii
    - P. aeruginosa

- Growth on CPO screening media
  - $n=21$

- Vitek2 meropenem MIC ≥0.5mg l$^{-1}$
  - $n=60$

- Routine clinical specimens

- CPO screening specimens

- Further investigations:
  1. Meropenem E-test
  2. ChromID CARBA SMART plate from same inoculum

- If any of:
  1. $\text{Rapidec Carba NP}$ test positive,
  2. Meropenem E-test MIC $>0.125$mg l$^{-1}$,
  3. Vitek2 meropenem MIC $≥0.5$mg l$^{-1}$

- Referral to reference unit

- Current study: In-house PCR for $\text{bla}_{\text{VIM}}$, $\text{bla}_{\text{IMP}}$, $\text{bla}_{\text{KPC}}$, $\text{bla}_{\text{NDM}}$ and $\text{bla}_{\text{OXA-48-like}}$ genes

- Growth on CPO screening media
  - $n=21$

- Further investigations:
  1. Rapidec Carba NP test
  2. Meropenem E-test
  3. Vitek2 susceptibility testing

- If any of:
  1. Meropenem E-test MIC $>0.125$mg l$^{-1}$,
  2. $\text{Growth on ChromID CARBA SMART plate}$ (Figure 2)

- Referral to reference unit

- Current study: In-house PCR for $\text{bla}_{\text{VIM}}$, $\text{bla}_{\text{IMP}}$, $\text{bla}_{\text{KPC}}$, $\text{bla}_{\text{NDM}}$ and $\text{bla}_{\text{OXA-48-like}}$ genes
Figure 2: Antibiogram of carbapenemase producing organisms

S: sensitive; R: resistant; I: intermediate; grey box: not tested

Table 1: Sample type from which CPO identified

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Number of CPO isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPO screening specimen*</td>
<td>19</td>
</tr>
<tr>
<td>Clinical specimen</td>
<td>20</td>
</tr>
<tr>
<td>Urine</td>
<td>11</td>
</tr>
<tr>
<td>Wound swab</td>
<td>3</td>
</tr>
<tr>
<td>Tissue sample</td>
<td>1</td>
</tr>
<tr>
<td>Sputum</td>
<td>3</td>
</tr>
<tr>
<td>Blood culture</td>
<td>2</td>
</tr>
</tbody>
</table>

Result of concomitant CPO screen if organism first detected on clinical specimen:

<table>
<thead>
<tr>
<th>Result</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>4</td>
</tr>
<tr>
<td>Negative</td>
<td>12</td>
</tr>
<tr>
<td>Not done</td>
<td>4</td>
</tr>
</tbody>
</table>

In 4 cases a CPO was identified from both a screening and clinical specimen.

* A total of 9057 screening specimens were examined during the study period.
Table 2: Clinical characteristics of cases where CPO detection represented infection (not colonisation)

<table>
<thead>
<tr>
<th>Organism (gene)</th>
<th>Relevant medical history</th>
<th>CPO infection details</th>
<th>CPO identification specimen</th>
<th>CPO screen result</th>
<th>Antimicrobial therapy</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. cloacae</em></td>
<td>Metastatic breast cancer, receiving carboplatin and paclitaxel chemotherapy</td>
<td>Concurrent <em>S. aureus</em> (MSSA) and <em>E. cloacae</em> bacteraemia</td>
<td>Blood culture</td>
<td>Negative</td>
<td>Flucloxacillin and temocillin</td>
<td>Survived</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>Gastric lymphoma, receiving R-CHOP chemotherapy. Recent neutropenic sepsis managed with piperacillin-tazobactam and gentamicin.</td>
<td>Bacteraemia</td>
<td>Blood culture</td>
<td>Negative</td>
<td>Ciprofloxacin and gentamicin</td>
<td>Survived</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>Back pain</td>
<td>Vertebrectomy surgical site infection</td>
<td>Wound swab</td>
<td>Positive</td>
<td>Co-trimoxazole</td>
<td>Survived</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>Resected low grade glioma.</td>
<td>Craniotomy surgical site infection with intra-cerebral collections (blood &amp; CNS sterile)</td>
<td>Wound swab</td>
<td>Negative</td>
<td>Co-trimoxazole, meropenem and gentamicin</td>
<td>Survived</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>Type 1 diabetes mellitus. Diabetic foot ulcer with underlying osteomyelitis.</td>
<td>Osteomyelitis and subsequent infected amputation site</td>
<td>Amputation tissue sample</td>
<td>Negative</td>
<td>Piperacillin-tazobactam, amikacin and colistin.</td>
<td>Survived</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>Initially admitted with intra-abdominal sepsis, successfully managed with piperacillin-tazobactam.</td>
<td>Nosocomial urinary tract infection</td>
<td>Urine</td>
<td>Negative</td>
<td>Trimethoprim</td>
<td>Survived</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>None</td>
<td>Urinary tract infection with sepsis</td>
<td>Urine</td>
<td>Negative</td>
<td>Ciprofloxacin</td>
<td>Survived</td>
</tr>
</tbody>
</table>
## SUPPLEMENTARY DATA

Supplementary table 1: Primer and probe sequences used for detection of carbapenemase genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence 5' – 3'</th>
<th>Concentration (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OXA-48-like</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>ATGCCTGTATCTATGCTTTTA</td>
<td>5</td>
</tr>
<tr>
<td>R</td>
<td>TGAGCATTCAAATTCAGAGC</td>
<td>5</td>
</tr>
<tr>
<td>P</td>
<td>CY5-CTACCGCAGGCTATTCGGAATAT</td>
<td>2</td>
</tr>
<tr>
<td><strong>KPC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>TGCGAGACCCAGGTGCACTTTT</td>
<td>6</td>
</tr>
<tr>
<td>R</td>
<td>CGCTCTATCGCGGATACCA</td>
<td>6</td>
</tr>
<tr>
<td>P</td>
<td>FAM-TTCCGTACGGCGGGCGCGCG</td>
<td>4</td>
</tr>
<tr>
<td><strong>NDM</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>CCTGATCAAGGCGACAGCAGCA</td>
<td>5</td>
</tr>
<tr>
<td>R</td>
<td>TGGCTCATACTACGATCATG</td>
<td>5</td>
</tr>
<tr>
<td>P</td>
<td>YAK-CCAAGTCGCTCGGCAATCTC</td>
<td>3</td>
</tr>
<tr>
<td><strong>VIM</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>GAGATTCCACGCACCTCTCTAGA</td>
<td>5</td>
</tr>
<tr>
<td>F2</td>
<td>GAGATTCCACGCACCTCTCTAGA</td>
<td>5</td>
</tr>
<tr>
<td>R</td>
<td>AATGCAGCAGCAGGATAG</td>
<td>5</td>
</tr>
<tr>
<td>P</td>
<td>TXR-ACGCAGTGCGGCTCGGTCAGT</td>
<td>2</td>
</tr>
<tr>
<td><strong>IMP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>GCCGGAATAGAGTGGCTTAATTCTC</td>
<td>10</td>
</tr>
<tr>
<td>R1</td>
<td>GAATTTTTAGCTTGACTTTAACGCTTT</td>
<td>5</td>
</tr>
<tr>
<td>R2</td>
<td>ATTTTTAGCTTGACCTTACGATT</td>
<td>5</td>
</tr>
<tr>
<td>R3</td>
<td>TTTGTAGCTTGACCTTATGCTTT</td>
<td>10</td>
</tr>
<tr>
<td>P</td>
<td>FAM-ATGCATCTGAATTAAC-MGB</td>
<td>2</td>
</tr>
</tbody>
</table>

F: forward; R: reverse; P: probe